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Altitudinal Variation in Digestive Tract Length in Yunnan Pond Frog (*Pelophylax pleuraden*)

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Abstract The digestive tract plays an important role in digestion and the acquisition of food energy. Understanding the impact of abiotic environments on digestive tract morphology is especially important for evolution of digestive tract across different environments. Here, we investigated altitudinal variation in digestive tract length in the Yunnan Pond Frog (*Pelophylax pleuraden*) across five populations ranging from 1413 m to 1935 m a.s.l. in Ningnan County, Sichuan province in western China. Frogs were collected during the breeding season, from 1–5 June 2012. Our results revealed that females had longer digestive tract and relative digestive tract (*i.e.* digestive tract length / body size) lengths in comparison to males, on average, but the differences between them decreased with increasing altitude. Digestive tract and relative digestive tract lengths increased with increasing altitude suggesting that a higher proportion of indigestible materials may be consumed at high-altitude sites and result in a relative increase in digestive tract dimensions.

Keywords altitudinal gradient, digestive tract, *Pelophylax pleuraden*, Ningnan county

1. Introduction

Organ size and mass often display differences among sexes or populations when organisms continuously cope with environmental fluctuation (Piersma and Lindstrom, 1997). Evolution of digestive tract morphology is affected by the abiotic environments due to morphology of digestive tract closely related to food habits in different environments (Wang *et al.*, 2003). Previous studies focused on latitudinal, altitudinal and seasonal variations in digestive tract and consumption of food quality in large-scale animals (small mammals, Naya *et al.*, 2008; Hammond *et al.*, 1999; birds, Leopold, 1953; Lewin, 1963; Gardarsson, 1971; Moss, 1972; Pendergast and Boag, 1973; Miller, 1975; McWilliams and Karasov, 2001; Piersma and Lindstrom, 1997; reptiles, Naya *et al.*, 2010) and amphibians (Secor, 2001; Naya and Bozinovic, 2004).

Habitats at high altitudes are generally associated with lower ambient temperature, more ultraviolet radiation and less food availability than habitats at low altitude. Taxa acclimating to lower ambient temperatures generally have a heavier gut mass (Hammond and Wunder, 1991; Hammond *et al.*, 1994; Koteja, 1996; Derting and Austin, 1998; Hammond *et al.*, 1999). For amphibians, changes in guts are not only affected by ambient temperature but also to other factors such as food type, reproductive events and sex (Naya *et al.*, 2003; Naya and Bozinovic, 2004). For example, Naya *et al.* (2009) found that the intestinal length of Andean toad (*Bufo spinulosus*) in northern Chile decreased with the increase of altitude due to the digestive tract dimensions increasing with the content of indigestible material in their natural diet.

The Yunnan Pond Frog (*Pelophylax pleuraden*) is distributed in the Yunnan Province, southwestern Sichuan Province and western Guizhou Province in China, ranging from 1150 m to 2300 m a.s.l., with habitats located in mountainous ponds, ditches and paddy fields (Fei and Ye, 2001). The reproductive period for this species lasts from June to July, and the food staple for the adult frogs are insects, accompanied with few small animals such as arachnids and snails (Fei and Ye, 2001; Luo, 2009). Population distribution, feeding habits,

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sexual size dimorphism, breeding ecology, heteromorphic comparisons and altitudinal variation in age and body size in *P. pleuraden* have been reported in recent years (Fei *et al.*, 2005; Zhou *et al.*, 2006; Zhou *et al.*, 2007; Luo, 2009; Lou *et al.*, 2012), but less research has been conducted on the variation in digestive tract lengths along altitudinal gradient. Here, we tested altitudinal variations in digestive tract length in *P. pleuraden* along altitudinal gradient by comparing the digestive tract lengths among five populations.

2. Material and Methods

2.1 Study area We sampled frogs from five different altitudes situated in Ningnan County in south-western Sichuan: 1413 m (27°10.72′ N, 102°36.27′ E), 1585 m (27°10.13′ N, 102°36.40′ E), 1785 m (27°09.73′ N, 102°35.97′ E), 1834 m (27°09.48′ N, 102°36.25′ E), 1935 m (27°10.29′ N, 102°34.77′ E). The 1413 m and 1935 m sites for these frogs are paddy fields approximately 170 m in length, 20–50 m width and 100 m length, where the vegetation is characterized by Rice (*Oryza sativa*). The 1585 m site is a ditch with approximately 300 m length and 2.5 m width, where the vegetation is characterized by Silvergrass (*Miscanthus floridulu*). And the 1785 m and 1834 m sites are ponds approximately 3 m length, 2–6 m width and 10 m length, 6 m width separately, where the vegetation is characterized by tobacco (*Nicotiana tabacum*) and *Pinus massoniana*. Annual mean air temperature in Ningnan county is 19.3 °C and the annual mean air temperature in the county decreases with altitude (Figure 1; annual air temperature data is from Ningnan county annals).

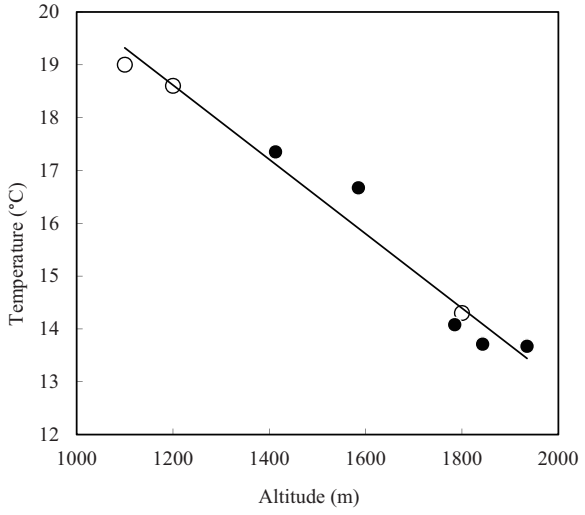

Figure 1 Annual mean air temperatures in Ningnan county including five sampling sites (solid dots) and three weather stations (open circles).

2.2 Samplings From 1–5 June 2012, a total of 168 adult males and 108 females were captured by hand at night using a flashlight in paddy fields, ditches and ponds (Table 1). Frogs were taken to the laboratory to study testes asymmetry, sperm competition and digestive tract length. After numbering the frogs and identifying their sex by direct observation of the vocal sacs in adult males and of the ova in adult females, they were stored in 10 % neutral buffered formalin following the procedure outlined by Simmons (1987). Six months later, the body size (*i.e.* snout-vent length: SVL) and the digestive tract lengths (*i.e.* the beginning of the esophagus to vent length) of specimens were measured to 0.01 mm with vernier calipers. Once the connected mesenteries were cut, the entire digestive tract was aligned along a caliper without stretching it, and the digestive tract length was recorded. We then dissected their stomach to observe the gastric contents. With the permission of Forestry Bureau of Ningnan County, we collected the frogs used in the study, following all the applicable institutional Animal Care Guidelines in China.

Table 1 Sample sizes and the number of empty stomachs and their ratios in five Yunnan Pond Frog (*Pelophylax pleuraden*) populations along the altitudinal gradient.

Altitude (m)	Sample size		Empty stomach number		Ratio of empty stomach (%)	
	Male	Female	Male	Female	Male	Female
1413	45	26	43	26	95.56	100.00
1585	55	18	53	18	96.36	100.00
1785	20	20	17	19	85.00	95.00
1834	22	16	21	15	95.45	93.75
1935	26	28	26	27	100.00	96.43
Total	168	108	160	105	95.24	97.22

2.3 Statistical analysis All data were analyzed by SPSS 17.0 (Statistical Product and Service Solutions Company, Chicago). We tested the differences of digestive tract lengths among populations with general linear models (GLMs) treating digestive tract length as dependent variable, altitude and sex as fixed factors and treating body size (SVL) as the covariate (to remove the effect of body size). The statistical test was set at two-tailed, and the significant level was set at $\alpha = 0.05$.

3. Results

Only 8 males and 3 females had food in their stomachs. A total of 160 males and 105 females, which had empty stomachs, were tested in our study (Table 1). SVL was significantly correlated with digestive tract length ($F_{1, 264} = 218.21$, $P < 0.001$). We compared the adjusted means

for digestive tract length between males and females. Females had longer digestive tracts (Table 2; GLMs: sex, $F_{1, 264} = 17.27$, $P < 0.001$) and relative digestive tracts (*i.e.* digestive tract length / SVL) than males in all populations (Figure 2), and the differences of mean relative digestive tract lengths between females and males decreased with altitudes (Figure 3).

The digestive tract lengths in *P. pleuraden* increased with altitude (GLMs: altitude, $F_{4, 264} = 7.09$, $P < 0.001$). To remove the effect of SVL, we displayed the variations of relative digestive tract length along altitude (Figure 2), showing that the relative digestive tract lengths increased with altitude.

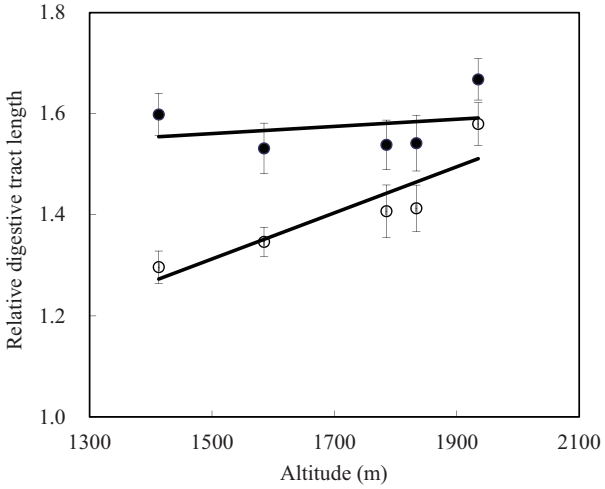

Figure 2 Variations in relative digestive tract lengths along the altitudinal gradient showing that the relative digestive tract lengths increase with altitude. Males = open circles; Females = solid dots. Points are means ± SE.

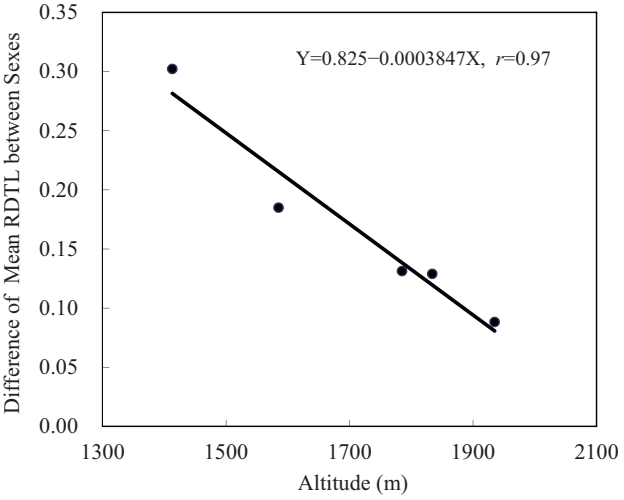

Figure 3 Differences of mean relative digestive tract length (RDTL) between females and males along the altitudinal gradient showing that differences between females and males decrease with the increase of altitude ($P = 0.006$).

4. Discussion

Studies on digestive flexibility are very common both in endotherms and ectotherms. For example, many species can adjust their digestive tracts when food habitats, seasonal changes and reproductive behaviors vary in different abiotic environments (Secor, 2001, 2005; Crump and Franklin 2003, 2005; Sabat *et al.*, 2005; Naya and Bozinovic, 2006; Naya *et al.*, 2009). In our study, digestive tract and relative digestive tracts differed among altitudes, suggesting that individuals from different altitude populations experience different abiotic environments related to food habitats.

Our data showed that females had longer digestive tracts and relative digestive tract in all *P. pleuraden* populations. Moss (1983) had found that different digestive abilities between males and females occurred within the same populations in Grouse and Ptarmigan, largely due to the difference in food quality. We did not know the food habits in *P. pleuraden* due to long-term starvation resulting in as much as 96.1 % empty stomach. However, according to the investigations of Luo (2009), differences in food quality between males and females can explain the differences of relative digestive tract lengths between the sexes. Differences in gut length per unit body weight between males and females may result from different energy requirements (Pulliainen, 1976). This pattern can also explain the differences of digestive tract lengths between the sexes in *P. pleuraden.*

Female gut attains a much greater mass than that of males during the period of high feeding activity due to reproductive events (*Rana temporaria* Juszczyk *et al.*, 1966; Naya *et al.*, 2003). The changes of digestive flexibility in males tend to be coupled with the annual feeding cycle, while it is mainly associated with reproductive cycle in females (Naya and Bozinovic, 2004). Consequently, changes in gut lengths are more obvious in females than males. However, we found that changes in digestive lengths and relative digestive lengths in males are more obvious than females (Table 2, figure 2). Future researches need to investigate the reason on changes in *P. pleuraden*.

The differences in digestive tract lengths between the sexes are associated with energy requirements during breeding activities (Naya and Bozinovic, 2004). We observed that the number of individuals participating in breeding activities (by observing the number of litters) significantly decreased with the increase of altitude. Therefore, the differences of mean relative digestive tract lengths between females and males decreasing with

Table 2 Variation in mean measurements of snout to vent length (SVL) and digestive tract lengths between sexes for five *Pelophylax pleuraden* populations. Values presented are absolute means ± SE for SVL and adjusted means ± SE (adjusted from GLMs analysis) for digestive tract lengths (Absolute values for digestive tract lengths are given in the Appendix Table S1).

Altitude (m)	SVL (mm)		Digestive tract length (mm)		N	
	Male	Female	Male	Female	Male	Female
1413	45.39 ± 0.72	52.02 ± 0.93	56.24 ± 1.46	68.38 ± 2.13	43	26
1585	41.12 ± 0.65	48.07 ± 1.12	60.61 ± 1.35	66.02 ± 2.31	53	18
1785	41.58 ±1.15	46.35 ± 1.09	62.80 ± 2.33	66.73 ± 2.21	17	19
1834	41.31 ± 1.04	44.75 ± 1.23	63.13 ± 2.10	68.17 ± 2.46	21	15
1935	38.44 ± 0.93	42.61 ± 0.91	70.60 ± 1.99	73.54 ± 1.84	26	27

increasing altitude should be associated with the number of individuals participating in breeding activities, despite no data on variation in energy need along the altitudinal gradient.

Our comparisons of digestive tract lengths from five *P. pleuraden* populations indicated that this species followed the pattern that populations with high altitude-sites have longer digestive tract and relative digestive tract lengths than populations with low altitude-sites. Similar results have been observed in Deer mice, *Peromyscus maniculatus* (Hammond *et al.*, 1999). For rodents, individuals living at higher altitudes associated with lower air temperatures have greater energy demands and have larger cardiopulmonary and digestive organs than those living at lower altitudes (Hammond *et al.*, 1999). Similarly, factors such as temperature can directly affect the food availability in amphibians (reviewed in Morrisson and Hero, 2003). Our data showed that the air temperature decreased with increasing altitudes. The increasing composition of the indigestible foods (e.g. low-quality, high-fiber food) and the decreasing digestible foods (e.g. animal-based foods) results in longer gut in animals (Sassi *et al.*, 2007). Decreasing plant-based foods and increasing animal-based foods in high altitude results in shorter gut length in *Bufo spinulosus* (Naya *et al.*, 2009). In our study, we inferred that high altitude sites may have more plant-based foods and less animal-based foods than low altitude sites, resulting in longer gut length in *P. pleuraden* in high altitude.

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Appendix

Table S1 The absolute mean measurements of digestive tract lengths between sexes for five *Pelophylax pleuraden* populations. Values given are means ± SD.

	Altitude (m)				
	1413	1585	1785	1834	1935
Male	59.06 ± 9.44	55.50 ± 9.91	58.54 ± 8.46	58.39 ± 8.73	60.51 ± 9.20
	(n = 43)	(n = 53)	(n = 17)	(n = 21)	(n = 26)
Female	83.51 ± 18.45	73.80 ± 15.26	71.30 ± 16.07	69.80 ± 19.61	71.20 ± 16.01
	(n = 26)	(n = 18)	(n = 19)	(n = 15)	(n = 27)